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Preparation and Characterization of Neoglycoprotein-Liposome Conjugates: A Promising Approach to Developing Drug Delivery Materials Applying Sugar Chain Ligands

糖鎖導入ドラッグデリバリー材料開発のためのネオ糖タンパク質・リポソームコンジュゲートの調製と機能評価

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Abstractπ

To translate the emerging insights into the functionality of the sugar code into applications in organic materials science, we have paid special attention to carbohydrate-protein(lectin) interactions. They are increasingly delineated to play an important role in biological recognition systems. Thus, the custom-made design of research tools and the examination of how to practically exploit them are becoming burgeoning research areas for producing new functional materials. We here report preparation and characterization of novel types of neoglycoprotein-liposome conjugates, and indicate applications by studying recognition functions of these tailored carriers with defined sugar part as the recognition function using a model system and *in vivo* experiments. Various types of neoglycoprotein-liposome conjugates were prepared according to a method including preparation of mixed micelles and then of liposomes, chemical coupling of neoglycoproteins to the characterized liposomes, and further sequential enzymatic glycosylation to refine the glycan part. The assays indicated carbohydrate-specific recognition functions of these neoglycoprotein-liposome conjugates. Monitoring of tissue distribution using Ehrlich solid tumor-bearing mice showed individual response of diverse tissues towards various types of applied neoglycoprotein-liposome conjugates harboring a series of sugar chain ligands including mono- and oligosaccharides. This type of carbohydrate-conjugated material is expected to find applications in basic glycoscientific research as well as in applied areas such as tissue-specific drug targeting materials.

要　約

糖鎖生物学的現象から有機材料への応用の道筋を見つけるために、我々は、生物の各種認識システムに重要な役割を果たしている糖鎖とタンパク質との相互作用に着目した。このような相互作用の応用研究がさまざまな分野で非常な興味を持たれており、その例として合成的研究や新しい機能性材料開発への応用などがある。本報告では、新しいネオ複合糖質としてのネオタンパク質・リポソームコンジュゲートの調製と特性評価を行い、そして、モデル系や生体系を使ったこれらネオ複合糖質の認識機能の評価結果を報告する。これらのコンジュゲートの調製は、次のような手順で行った。つまり、混合ミセルの調製、リポソームの調製、ネオ糖タンパク質のリポソームへの化学的カップリング、そして、段階的酵素反応によるグリコシレーションである。これらのコンジュゲートの糖鎖特異的な認識機能については、モデル系や*in vitro*のアッセイで評価した。また、*in vivo*のアッセイでは、エーリッヒ固形癌の担癌マウスを使って評価した。その結果、単糖やオリゴ糖を導入した各種のリポソームにおいて糖鎖に特徴的な体内動態結果が得られた。これらの新規な糖鎖導入材料は、組織特異的なドラッグターゲティング用材料とともに、糖鎖の分子認識研究などにも応用できると考えられる。

A. Introduction

Carbohydrates have been generally viewed as energy-storage substances and physical maintenance materials, as exemplified by glycogen and cellulose, respectively. By appreciating the

A．はじめに

糖質は、従来グリコーゲンやセルロースに代表されるようなエネルギー源や構造物質として論じられることが多かった。しかしながら、オリゴ糖鎖の構造と機能の多様性のおか

distinct structural talents of saccharides, this view has changed markedly. In fact, it has become recognized in recent years that carbohydrates represent informational biomolecules establishing a code system which can be referred to as the "third alphabet of life" to signal the inherent capacity of oligosaccharides (words) to store and transmit information. A large number of biological events such as cell-cell recognition, adhesion, growth regulation, and inter- and intracellular routing have been shown to involve carbohydrates of glycoproteins and glycolipids for correct and efficient molecular interplay. Today, proteins, which participate as receptors in such interactions and specifically recognize the structural features of carbohydrate ligands, i.e., animal lectins, are increasingly being discovered to play salient roles in biological carbohydrate-protein recognition systems(1–10).

With this background it comes as no surprise that carbohydrate-protein interactions are attracting increasing interest in synthetic studies as a step towards evaluating practical applications. With a complete book devoted to this issue (11), the term "neoglycoconjugate" used to classify a family of synthetic materials, in which functional carbohydrate groups are linked to proteins, lipids, polymers, or solid matrices for providing desirable biochemical and physicochemical properties, has taken a firm hold. Neoglycoconjugates include neoglycoproteins, neoglycolipids, clusters, dendrimers, telomers, glycopolymers, and also glycoliposomes. These synthetic glycoconjugates have been designed to produce new substances and materials, such as biochemical probes, vaccines, inhibitors of cell adhesions, ligands in affinity chromatography, diagnostic reagents, therapeutic reagents, gene targeting carriers, and drug targeting devices(11–18).

As a graphic example for a route to turn the functionality of endogenous lectins into future applications based on organic materials technology, we have developed a new type of neoglycoconjugate, neoglycoprotein-liposome conjugates. We here report on the current state of our research in this area, including establishment of a method to prepare an innovative type of carbohydrate-conjugated liposomal material and their applications for studying carbohydrate-protein recognition functions in a model system *in vitro* and *in vivo*. This approach is aimed at the design of drug delivery materials applying sugar chain ligands.

B. Preparation and Characterization of Neoglycoprotein-Liposome Conjugates

The experimental procedure for preparation of liposomes and for coupling of neoglycoproteins to liposomes is a modified method, which is an adaptation of the controlled detergent dialysis method for liposome preparation by Zumbuehl and Weder(19) and the two-step method for protein coupling by Heath *et al.*(20). The preparation procedure is divided into two parts that are further subdivided as follows. First, formation of

げで、このような概念は大きく変化してきた。最近では、糖鎖は情報分子として認知され、オリゴ糖鎖を単語とする"生体の第3番目のアルファベット"としてのコードシステムを構成している。それは、細胞間の認識や接着、増殖制御、細胞内外の情報伝達などの過程において、糖タンパク質や糖脂質の糖鎖がシグナル分子として働いているということが明らかとなってきたからである。そして、そのような相互作用においてレセプターとして糖鎖の構造を認識しているタンパク質がレクチンであり、それらの顕著な役割が数多く発見されつつある(1–10)。

このような生物学的認識機構に糖質が関与していることがわかってくるに従い、それらの糖質·タンパク質相互作用が合成や応用研究において注目されてきている。そして、近年この種の合成物質の一群をネオ複合糖質と称しており(11)、それらの物質では機能性の糖鎖がタンパク質、脂質、ポリマー、固体マトリックスなどに結合され、必要な生化学的あるいは物理化学的な特性を有するように作られる。このようなネオ複合糖質として知られているものとしては、ネオ糖タンパク質、ネオ糖脂質、クラスター、デンドリマー、テロマー、グリコポリマー、グリコリポソームなどがある。このような合成の複合糖質は、次のようなさまざまな目的に適するように設計されている。例えば、生化学的なプローブ、ワクチン、細胞接着インヒビター、アフィニティークロマト担体用リガンド、診断薬、治療薬、遺伝子ターゲティングキャリアー、ドラッグターゲティング材料などである(11–18)。

このような糖鎖生物学的な機能の有機材料開発への応用の道筋を見つけるために、我々も糖鎖とレクチンとの相互作用に着目し、新しいタイプのネオ複合糖質であるネオ糖タンパク質・リポソームコンジュゲートの開発を試みた。ここでは、我々のこれまでの初期段階の研究成果を報告する。まず、糖質とリポソームとの結合体の調製方法、そして、それらのモデル系や生体系での認識反応性を調べた。これらの結果は、糖鎖を導入した新しいタイプのドラッグデリバリー材料の開発に寄与できると考えられる。

B．ネオ糖タンパク質・リポソームコンジュゲートの調製とその特性

リポソームの調製とネオ糖タンパク質のリポソームへのカップリングは、それぞれZumbuehl&Weder(19)とHeathら(20)の方法に従って次のように行った。まず、リポソームの調製は、コール酸透析法によって行った。a) 脂質と界面活性剤の混合ミセルの調製、そして、b)混合ミセルの透析を行った。次にネオ

liposomes by a cholate dialysis method including a) preparation of lipid/detergent mixed micelles and b) dialysis of the mixed micelles. Second, covalent coupling of neoglycoproteins to liposomes by a two-step reaction method, including a) periodate oxidation of gangliosides in the liposome membrane and b) coupling of neoglycoproteins to oxidized liposomes by reductive amination. According to the strategy of the procedure, as shown in Fig.1, a series of neoglycoproteins could be coupled to liposomes, which resulted in various types of neoglycoprotein-liposome conjugates displaying monosaccharide and disaccharide sugar chains.

In order to analyze the homogeneity and stability of liposomes, size distribution is an important characteristic among

糖タンパク質のリポソームへのカップリングは、以下の2段階反応によって行った。a) リポソーム膜上のガングリオシド部分の過ヨウ素酸酸化、および b) 還元的アミノ化反応による酸化リポソームへのネオ糖タンパク質のカップリングである。その反応フローの一例を図1に示した。このような手法によって一連のネオ糖タンパク質をリポソームに結合することができ、単糖や二糖を有する多種多様なネオ糖タンパク質・リポソームコンジュゲートが得られた。リポソームの純度や安定性を見るために粒径サイズ分布を調べることが非常に重要である。その方法とし

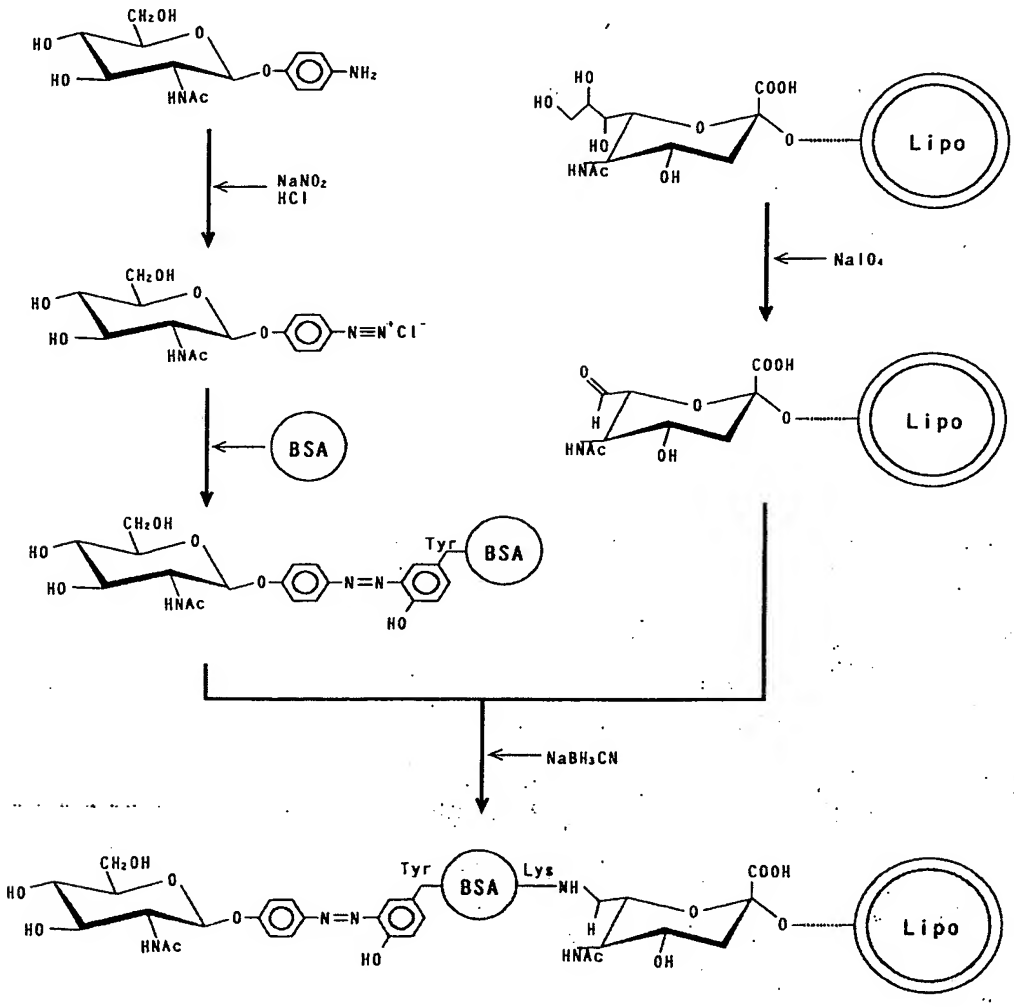

Fig. 1. Outline of the reaction pathway for neoglycoprotein-liposome conjugation.
Preparation of N-acetylglucosaminylated bovine serum albumin(BSA)-coupled liposomes(Lipo) was chosen as an example.

several physical properties. Gel-permeation chromatography-(GPC), scanning electron microscopy(SEM), and dynamic light scattering(DLS) were applied for size characterization of liposomes. Fig.2 ascertains the homogeneity of the liposome population harboring dipalmitoyl phosphatidylcholine (DPPC), Chol, dicetyl phosphate (DCP) and gangliosides at a molar ratio of 35:45:5:15. This type of liposome was stable and did not change its property after storage at 4Åéfor several months. Accordingly, this preparation was selected for conjugation of neoglycoproteins. Characterization of neoglycoprotein-coupled liposome preparations was carried out using high-performance GPC, as well as by SEM and DLS. The results revealed that the liposome preparations examined were invariably homogeneous and stable for several months. To analyze the *in vivo* stability of mannosylated BSA-coupled liposomes, the liposome suspension was injected into the tail vein of a male mouse, blood was collected after three hours, and serum was prepared. The serum was purified using an ultrafiltration cell fitted with a 0.03-mm polycarbonate membrane, which resulted in recovery of mannosylated BSA-coupled liposomes. Monitoring of the morphology of the mannosylated BSA-bearing liposomes by SEM before and after *in vivo* treatment did not reveal any difference between the liposome prior to injection and the recovered liposome. These results substantiate that our liposomal preparations are stable *in vivo* as well as *in vitro*. This stability allows their application for further studies including their use as drug delivery carriers.

て、ゲル濾過クロマト法 (GPC)や走査型電顕 (SEM)や動的光散乱法 (DLS)などを使った。図2にジパルミトイルホスファチジルコリン(DPPC)、コレステロール、ジセチルリン酸 (DCP)、ガングリオシドのモル比 35：45：5：15のタイプのリポソームの純度測定結果を示す。このリポソームは4℃で数ヶ月保存しても安定であったので今後のネオ糖タンパク質結合用の原料とした。今後用いるネオ糖タンパク質結合リポソームの特性は、SEMやDLSと共に、GPCによっても検定した。また、マンノース単糖を導入したリポソームの*in vivo*での安定性をマウスを使って調べた。このマンノース導入リポソームをマウスに静注し、3時間後に採血して血清を調製した。そして、孔径 0.03μmの膜を用いて限外濾過を行いリポソームを精製し回収した。そのSEM観察の結果、このマンノース導入リポソームの形態は*in vivo*での3時間処理・回収前後においても変化がなかった。これらの実験により、我々のリポソーム標品は*in vitro*と共に*in vivo*においても安定であることが示された。従って、これらのコンジュゲートはドラッグデリバリーキャリアーのための研究に使用できることがわかった。

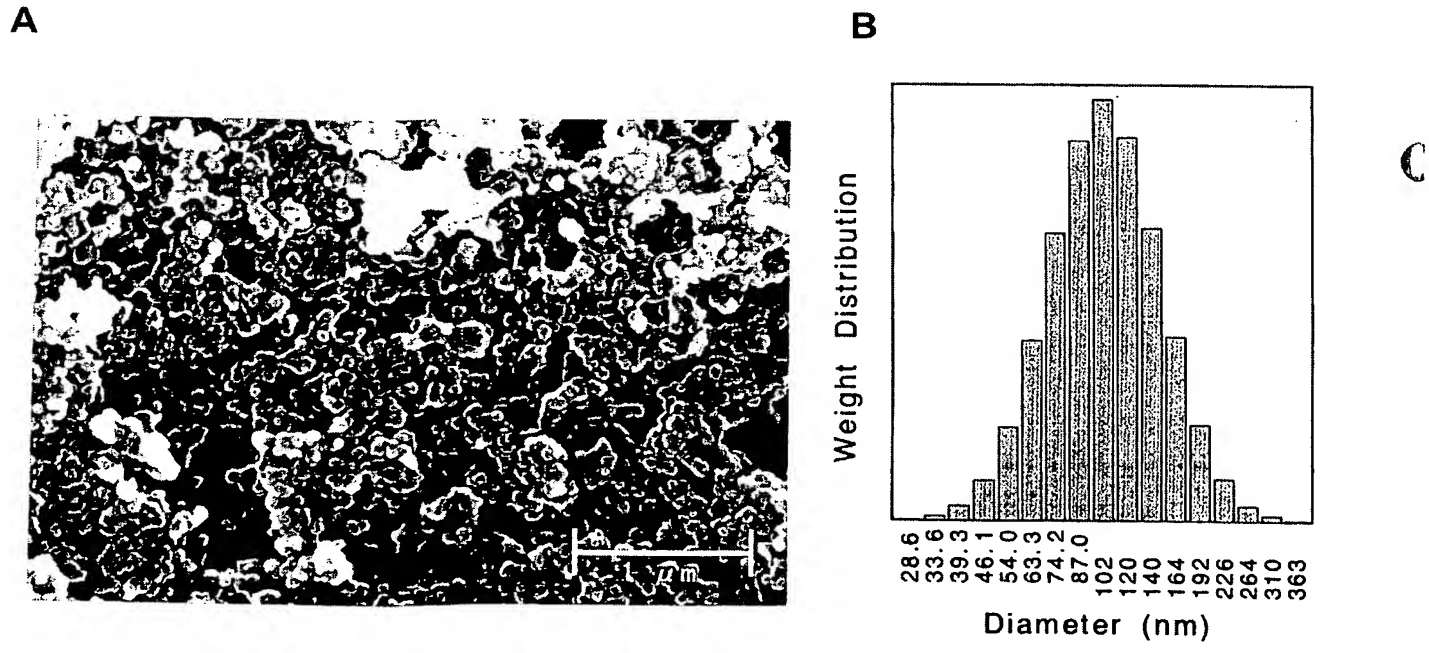

Fig. 2. Scanning electron micrograph (A; magnification, ×19,000), and bar histogram of dynamic light scattering (B) of the liposome population prepared following the strategy shown in Fig.1.

C. Binding Analysis of Neoglycoprotein-Liposome Conjugates to Lectin-Liposome Conjugates and Biodistribution of Radiolabeled Neoglycoprotein-Liposome Conjugates

In order to obtain insight into the understanding of the multivalent interactions between neoglycoprotein-liposome conjugates as ligands and lectins conjugated on the membrane surface of lipid vesicles as receptors, a series of lectin-conjugated lipid vesicles were prepared, and binding studies were performed by applying these model systems consisting of different ligands and receptors, as shown exemplarily in Fig.3A. To analyze the binding process using, for example, mannosylated BSA-bearing liposomes and Concanavalin A (Con A)-bearing liposomes, the time course of the agglutination was followed by measuring the change in the turbidity, together with inhibition assay using hapten sugars, and observation by DLS and SEM. The combined results demonstrated that the increase in turbidity was due to the formation of vesicle clusters that resemble cell aggregates, and that the agglutination was caused by the carbohydrate-dependent cross-linking between mannosyl residues of mannosylated BSA-conjugated liposomes and Con A lectins of Con A-conjugated liposomes, as shown in Fig.3B.

To assess binding or uptake of liposomal conjugates to individual tissues of Ehrlich solid tumor-bearing mice in the more complex *in vivo* situation we determined the biodistribution of

C．ネオ糖タンパク質・リポソームコンジュゲートの結合実験と体内動態

膜面上での糖鎖とレクチンとの多価性の相互作用を解析するために、次のような実験を行った。ネオ糖タンパク質リポソームコンジュゲートの調製と共に、レクチンをリポソームに結合したコンジュゲートも調製した。そして、これら2種類のリポソームを図3Aのようなシステムで結合反応を行った。その結合反応の時間経過は、反応液の濁度変化で調べた。またその変化をDLSやSEMによっても観察した。さらに、その糖特異性をハプテン糖を用いた阻害実験によっても確かめた。その結果、例えば、図3Bに示されるように、これら2種類のリポソームによる濁度変化は細胞間の凝集のようなベシクル間のクラスター形成によるものであることがわかった。またこれらの反応は、糖鎖であるマンノース、およびレクチンであるCon -Aとの間の特異的な反応であることが実証された。さらに、複雑な生体システムでの*in vivo*アッセイを行った。具体的には、エールリッヒ固形癌を担癌したマウスを用いて、^{125}Iで標識した糖タンパク質リポソーム複合体を尾静注し、6時間後における組織分布を調べた。その結果、図4に示すように、各種のネオ糖タンパク質結合

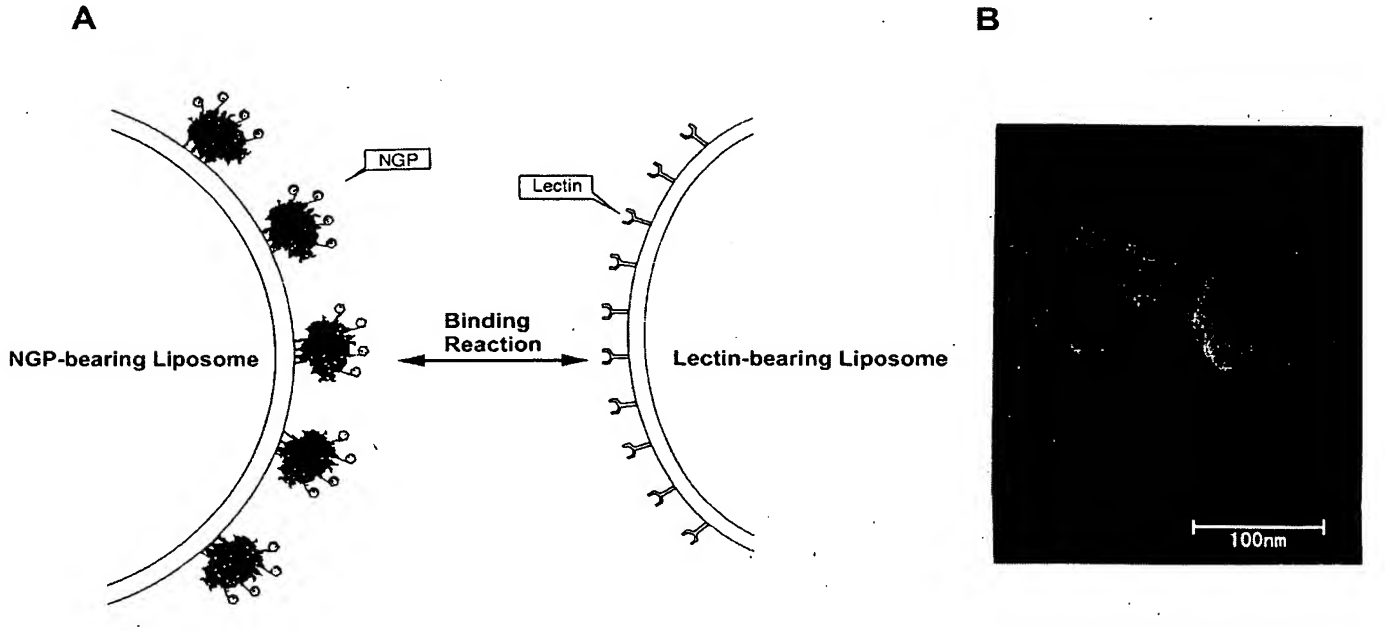

Fig. 3. Schematic illustration of the reciprocal recognition system based on carbohydrate-protein interactions of neoglycoprotein (NGP)-bearing liposomes and cognate-lectin-bearing liposomes(A), and scanning electron micrograph of the reaction mixture, which was incubated for 20 min to allow formation of lectin-ligand association (B; magnification, × 200,000).

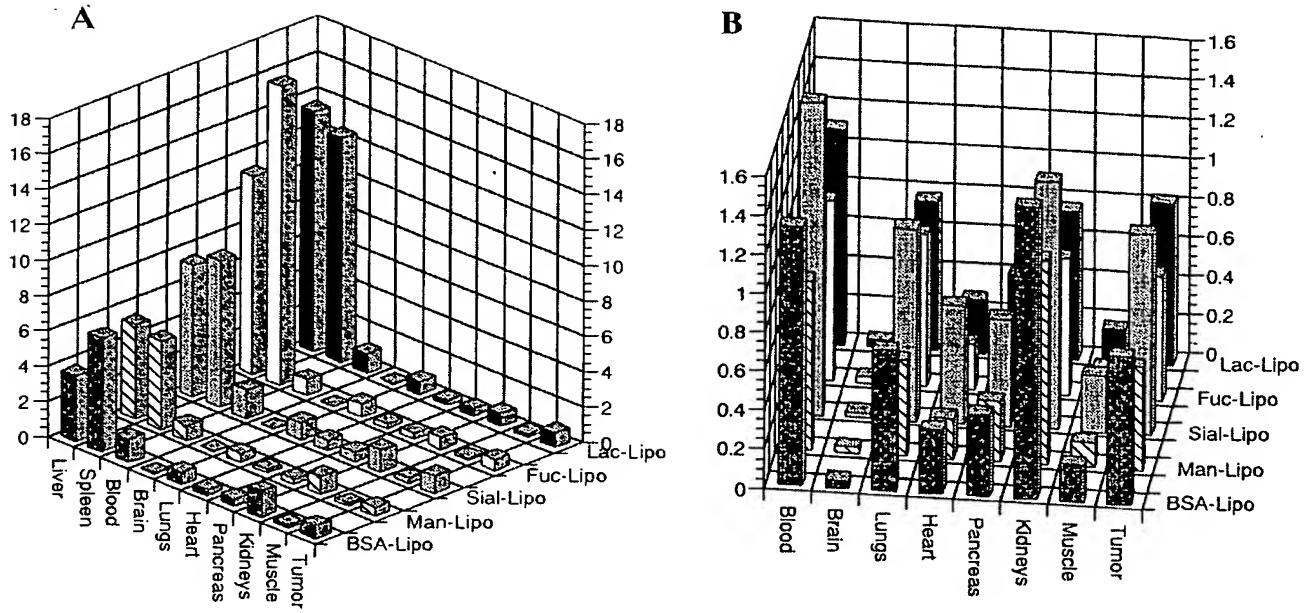

Fig. 4. Biodistribution of four types of ^{125}I-labeled neoglycoprotein-coupled liposomes and ^{125}I-labeled BSA-coupled liposomes in Ehrlich solid tumor-bearing mice after 6h, expressed as percentage of the injected dose per g of tissue. (A) Data of all tissues examined, and (B) data of tissues excluding liver and spleen. Each result represents the mean of quadruplicates. Abbreviations: BSA, bovine serum albumin; Man, D-mannose; Sial, N-acetyl-D-neuraminic acid; Fuc, L-fucose; Lac, lactose.

the [^{125}I]neoglycoprotein-coupled liposomes 6 hr after injection into the tail vein of the mice. As shown in Fig.4, the individual response on the level of organ content showed differences among different types of neoglycoprotein-conjugated loposomes. These data constitute the basis for further refinements of the carbohydrate ligands of suitable neoglycoprotein-conjugated liposomes to allow their potentially rational application as drug-targeting devices. It is noteworthy that Kole et al.(21) have successfully applied mannosylated neoglycoprotein-conjugated liposomes, which were prepared according to our method, as macrophage-specific drug carrier in a therapeutic model of visceral leishmaniasis, a macrophage-associated parasitic disease.

D. Preparation and Biodistribution of Four Types of Neoglycoprotein-Liposome Conjugate with Different Levels of Structural Complexity of the Sugar Chain Ligands

By applying sequential chemoenzymatic steps, four types of glycosylated bovine serum albumin (BSA)-liposome conjugates were prepared according to the following procedure. First, N-acetylglucosaminylated BSA was synthesized and this was then chemically conjugated to liposomes, which resulted in N-acetylglucosamine (GlcNAc)-bearing BSA-liposomes. GlcNAc-bearing BSA-liposomes were enzymatically galactosylated by

リポソームは、種々の組織に異なる割合で分布することがわかった。これらの結果は、本標品の糖鎖リガンドを今後更に改良を行うことにより、合理的なドラッグターゲティングデバイスとしての応用の可能性を示すと考える。さらに、注目すべきこととして、我々が開発したこのような手法を用いて、Koleら(21)のグループが次のような実験結果を発表した。まず、マンノースを導入したネオ糖タンパク質結合リポソームを調製し、これらがマクロファージ関連寄生虫病であるリーシュマニア症という感染症において、マクロファージ特異的ドラッグデリバリー機能により治療効果を発揮することを示した。

D. 糖鎖構造の異なる4種類のオリゴ糖鎖導入リポソームコンジュゲートの調製と体内動態

段階的な化学的・酵素的合成方法によって4種類の糖鎖を導入したリポソームコンジュゲートを調製した。まず、*N*-アセチルグルコサミン結合BSAを合成し、これをリポソームに化学的に結合して、*N*-アセチルグルコサミン導入BSA結合リポソームを調製した。次に、β1,4ガラクトシルトランスフェラーゼ酵素を用いてリポソーム上のGlcNAcをガラクトシル化した。そして、この産物LacNAc導入リポソームをさらにα2,6シアリルト

using β1,4-galactosyltransferase, and the products, i.e. N-acetyllactosamine (LacNAc)-bearing liposomes, were sialylated by α2,6-sialyltransferase treatment to prepare 6'sialyl-N-acetyllactosamine-bearing liposomes according to the reaction scheme outlined in Fig.5. The LacNAc-bearing BSA-liposomes were also used to prepare Lewis x trisaccharide-bearing BSA-liposomes by treatment with α1,3-fucosyltransferase in the presence of substrate following a similar scheme. Coupled protein/total lipid ratios of the four types of liposomes were determined to be between 0.22 and 0.25g/g, and average numbers of BSA molecule per liposome, calculated by using estimated areas of protein and liposome surfaces, were about 500. The four types of glycosylated BSA-liposome conjugates were homogeneous in size as judged by GPC and did not change their property after storage at 4°C for several months. Their characteristics, together with the previous *in vivo* stability data of related neoglycoprotein-bearing liposomes, are essential for their applications as drug-

ランスフェラーゼ処理して、6'シアリル-*N*-アセチルラクトサミン導入リポソームを調製した(図5参照)。また、同様のLacNAc導入リポソームからα1,3フコシルトランスフェラーゼで処理をすることによりルイスX三糖を導入したリポソームを調製した。このようにして得られた4種類のリポソームについて、結合タンパク質と全脂質量を定量した結果、比率は0.22 ～ 0.25 g/gの範囲であった。更に、タンパク質とリポソームの表面積から計算することにより、1つのリポソームに結合した糖タンパク質の数が約500程度であることが推定された。また、4種類の糖鎖導入リポソームコンジュゲートのGPC測定により、粒径分布が均一であることがわかった。そして、4℃に数ヶ月間保存した後もその特性は変わらないことがわかった。このような特性は、前述の*in vivo*の安定性データと共に、ドラッグターゲティングデバイスへの応用にとって非常に重要であると考える。次に、これ

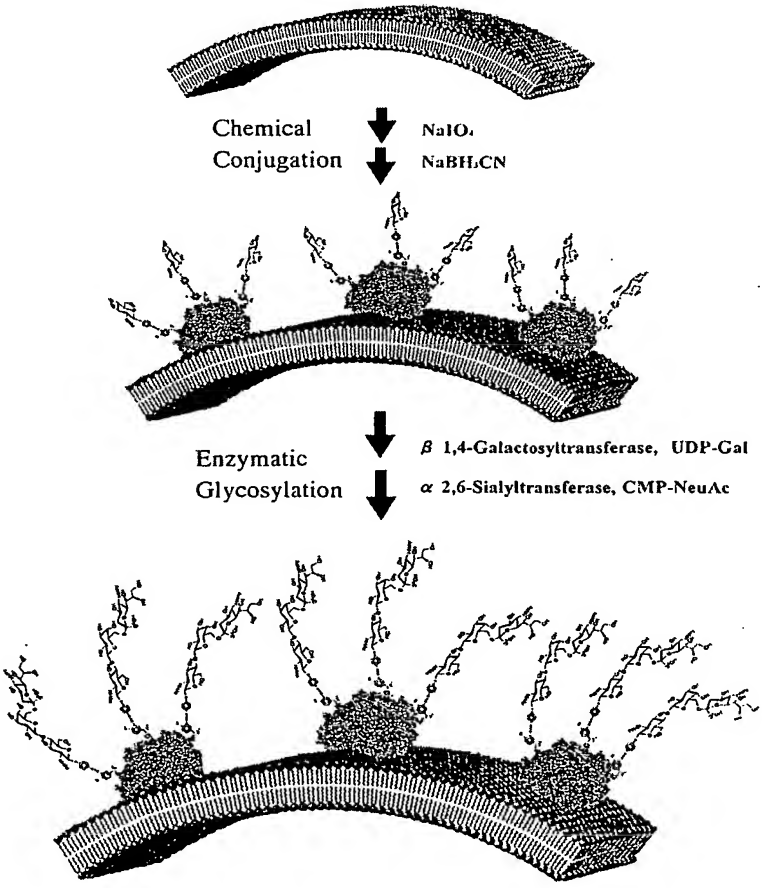

Fig. 5. Reaction scheme for synthesis of neoglycoprotein-liposome conjugates bearing 6'-sialyl-N-acetyllactosamine trisaccharides.

targeting devices.

In order to assess the tissue-specific uptake of various glycosylated BSA-bearing liposomes, we determined the biodistribution of four types of ^{125}I-labeled neoglycoprotein-bearing liposomes 3hr after injection into Ehrlich solid tumor-bearing mice (Fig.6). The individual response on the level of organ content showed considerable differences among this panel of neoglycoprotein-bearing liposomes. Relative to GlcNAc-bearing liposomes, the uptake of LacNAc-bearing liposomes was increased in mice clearly for liver, spleen and lung, and slightly for tumor. Remarkably, the uptake of 6'sLacNAc-bearing liposome was decreased distinctly for spleen, the uptake for other tissues being almost maintained. On the other hand, the uptake of Lewis x-bearing liposomes was increased especially for liver, spleen and lung. These results emphasize the importance of structural remodeling of carbohydrate ligand chains within the production of tissue-specific drug delivery devices.

E. Perspectives

In this report, we have summarized results from the current stage of our research, which aims at design and construction of recognition functions based on carbohydrate-protein interactions, and which also aims at finding their possible applications in the field of biosensing, drug design, membrane engineering, and especially drug delivery devices.

らの糖鎖導入リポソームの組織特異的分布を調べるために、^{125}Iでラベルしたリポソーム4種類をエールリッヒ固形癌担癌マウスに尾静注し、3時間後にその組織分布を測った(図6参照)。その結果、組織あたりの各種リポソームコンジュゲートの分布状態はかなり異なることがわかった。例えば、LacNAc導入リポソームの組織分布は、GlcNAc導入リポソームのそれと比べて、肝臓、脾臓、肺において明らかに増加しており、腫瘍においてもわずかに増加していた。更に注目すべきことに、6'シアリルLacNAc導入リポソームの組織分布は、LacNAc導入リポソームのそれと比べて、ほとんどの臓器で分布が同じであるが、脾臓においてのみ特徴的に減少していた。一方、ルイスX糖鎖導入リポソームの組織分布は、肝臓、脾臓、肺において、他種のものより更に増加するということがわかった。これらの結果により、組織特異的なドラッグデリバリーデバイスを調製するには、糖鎖リガンドの構造をリモデリングすることが重要であるということがわかってきた。

E. 展　望

我々は糖鎖とタンパク質との脂質膜面上での2次元的認識機能の研究や、バイオセンシング、ドラッグデザイン、膜工学、そして、とりわけドラッグデリバリーデバイスなどへの応用を

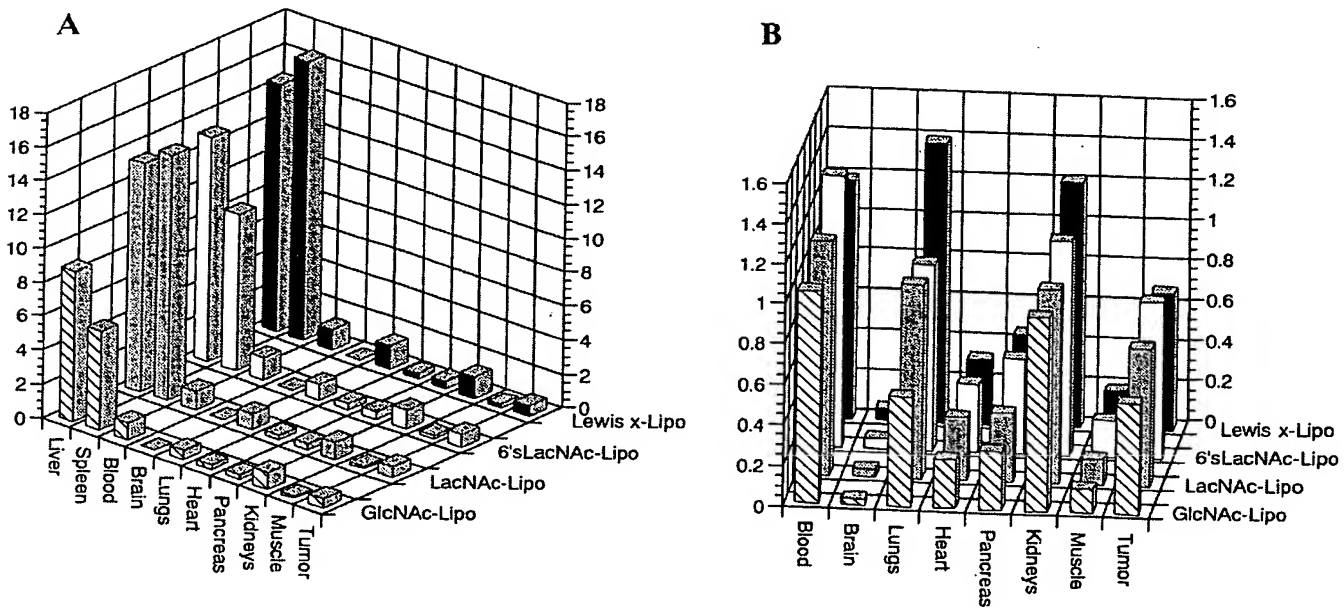

Fig. 6. Biodistribution of four types of ^{125}I-labeled neoglycoprotein-liposome conjugates in Ehrlich solid tumor-bearing mice after 3h, expressed as percentage of the injected dose per g of tissue. (A) Data of all tissues examined, and (B) data of tissues excluding liver and spleen. Each result represents the mean of quadruplicates. Abbreviations: GlcNAc, N-acetyl-D-glucosamine; LacNAc, N-acetyllactosamine; 6'sLacNAc, 6'-sialyl-N-acetyllactosamine; Lewis x, Lewis x trisaccharide.

cations in such fields as biosensing-, drug design- and membrane-materials, and especially drug delivery devices. Presently, we have established a method to prepare a series of neoglycoprotein-liposome conjugates. Analyses of these preparations proved homogeneity and stability. The *in vivo* stability was also satisfactory, which fulfilled a prerequisite for considering applications in biological systems. A model and *in vitro* assays demonstrate that this type of neoglycoprotein-bearing liposomes has carbohydrate-specific activities and is useful as a cytochemical probe. A biodistribution assay indicates the potential usefulness of neoglycoprotein-liposome conjugates as efficient drug-targeting devices which exploit cellular functions of carbohydrate-binding proteins.

Whereas a considerable level of expertise and experience in developing carbohydrate-mediated drug delivery systems based on glycolipid-bearing liposomes has been attained, the custom-made design and applications of glycoprotein-bearing liposomes are less explored(22–28).In a recent commentary on receptor-mediated gene delivery it was stated that "the idea of a ligand is generally associated with a polypeptide... we have almost been oblivious to the potential of sugars as specific ligands for the specific delivery of drugs or genes, which explains why the field of 'glycotargeting' is in its infancy"(29). As a step to address this issue, efforts are directed to design and construct high-functional neoglycoprotein-liposome conjugates which display complex oligosaccharide chains on the liposomal surface. An example of our ongoing experiments aims to synthesize a new type of neoglycoprotein-liposome conjugate containing sialyl Lewis X sugar chains using chemical and enzymatic procedures. First, N-acetylglucosaminylated BSA-coupled liposomes were prepared according to the procedure described in this report. Then, three-step enzymatic glycosylation was performed using β1,4 -galactosyltransferase, α 2,3-sialyltransferase, and α1,3-fucosyltransferase as catalysts, which resulted in a new type of oligosaccharide-conjugated material. By applying this kind of approach or by developing other techniques, we expect to provide new insights into the underlying mechanisms of carbohydrate-protein interactions on membrane surface, and to eventually define applications in materials technology such as drug delivery systems.

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目的として研究してきた結果の一部を本稿に報告した。そして、ネオ糖タンパク質リポソームコンジュゲートの調製法、その産物の特性が均一で安定であること、*in vivo*においても安定であり、生体系での応用にも適していること、そして、*in vitro*のアッセイにより糖鎖特異的に働くこと、従って、例えば細胞化学的なプローブとしても使えることなどを示した。また、*in vivo*での組織分布結果から、この種のコンジュゲートは糖鎖機能を利用したドラッグデリバリーデバイスとしても応用できる可能性が明らかとなった。糖鎖を介したドラッグデリバリーシステムの研究開発は、糖脂質を導入したリポソームについてかなりの数の研究が知られているが、糖タンパク質を導入したリポソームのオーダーメイド設計や応用に関する研究はほとんどなされていない(22–28)。レセプター介在型遺伝子送達に関する最近の論評によると(29)、これまでの研究では、薬物や遺伝子の特異的な送達のためのリガンドとしては、ポリペプチドが使われることが多く、糖鎖の可能性についてはほとんど忘れられてきた。このことが、このグリコターゲティング研究の遅れの原因であると思われると述べられている。この課題を解決するために、我々はリポソーム膜面上に複合糖質オリゴ糖を導入して、高機能なネオ糖タンパク質リポソームコンジュゲートを設計・調製することを試みている。現在進行中の実験の一例を以下に示すと、例えばそれはシアリルルイスXタイプの糖鎖を有するコンジュゲートであり、その調製法は、GlcNAc導入リポソームをβ1,4ガラクトシルトランスフェラーゼ、α2,3シアリルトランスフェラーゼ、そしてα1,3フコシルトランスフェラーゼの3段階の酵素的グリコシレーションにより、新しいタイプのオリゴ糖導入コンジュゲートを調製できた。今後、本稿で述べたような方法やその他の方法を開発して、脂質膜面上での糖鎖とタンパク質との相互作用の機構を解析したり、その成果をドラッグデリバリーシステムのような材料開発に応用したりできることを期待している。

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